The Relationship of Tumor Necrosis to White Blood Cell Changes in the Hamster*

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In a previous publication it was pointed out that definite relationships existed between the growth curve of a 100 per cent transplantable methylcholanthrene-induced hamster sarcoma and several blood cell factors (12). As the tumor grew in the hamster cheek pouch, characteristic alterations were noted in the levels of hemoglobin, red and white blood cells, differential white blood cell counts, hematocrits, sedimentation rates, and in platelet determinations.

It was speculated that the elevated white blood cell counts and sedimentation rates and the reversed neutrophil to lymphocyte ratios were associated with the presence of tumor necrosis, which occurred at an early stage in the growth of the tumor but did not become apparent externally until a later time in the tumor growth cycle. It was the purpose of this study to determine whether these alterations in the white blood cells and sedimentation rates could be produced by the products of tumor necrosis in the absence of viable tumor.

MATERIALS AND METHODS

One hundred and ten golden hamsters (Mesocricetus auratus) of both sexes, 8-10 weeks old, fed Purina Laboratory Chow and water ad libitum were used in this study. The tumor employed was a methylcholanthrene-induced spindle-cell sarcoma of the hamster that had been serially propagated in the cheek pouch of the hamster (7). The technic of transplantation, the method of tumor observation, and the tumor growth curve characteristics have been previously described (7, 11, 12).

Tumors containing large areas of necrosis were removed from hamster cheek pouches after 35-50 days. The necrotic material was dissected from the remaining tumor mass, weighed, and ground in a mortar. It was then suspended in 0.85 per cent saline, with 30 cc. of saline used for each gram of necrotic material. The suspension was first passed through a Buchner funnel and then through an N Berkefeld filter into a sterile flask. A sample of the last filtrate was streaked on nutrient agar to check its sterility.

The viable tumor, dissected from the necrotic portion of 35-50-day tumors, was weighed and ground in a mortar. Each gram of non-necrotic tumor was then suspended in 30 cc. of normal saline. It was filtered through a Buchner funnel and then through an N Berkefeld filter into a sterile flask. A sample of the last filtrate was streaked on nutrient agar.

Blood obtained by cardiac puncture was used for all determinations, and standard hematologic technics were employed to determine the total and differential white blood cell values and the sedimentation rates. The average of two determinations, done 10 days apart before any intraperitoneal injections, was used as the normal value in any one animal.

Forty-five hamsters were given injections intraperitoneally of 0.5 cc. of the sterile, cell-free extract of necrotic tumor tissue every day for 50 days; 50 hamsters were given injections intraperitoneally of 0.5 cc. of sterile, cell-free extract of non-necrotic tumor every day for 50 days; and fifteen hamsters received daily intraperitoneal injections of 0.5 cc. of sterile saline for 50 days. White blood cell values and sedimentation rates were determined in all three groups every 5th day.

All animals were sacrificed at 50 days. Complete autopsies were performed, and tissues were prepared for histologic study. Smears were made from femoral bone marrow and stained with a combination of Wright's stain and Giemsa's stain. Blood cultures were drawn on all animals three times during the experiment.

RESULTS

The injected filtrate of necrotic material produced changes in the white blood cells and the
sedimentation rate that were similar to those found in hamsters bearing transplanted tumors (12), while the injection of normal saline produced no change from the normal. The animals that were injected with necrotic material showed a definite leukocytosis (Chart 1), and the average values were significantly greater than the control or normal values from the 20th day after injection to the conclusion of the experiment (P = 0.05).

Leukocytosis was noted 24 hours after the first injection, but a sustained elevation was not apparent until the 20th day.

Fifteen days after injection of the necrotic material a statistically significant increase (P < 0.01) in the average neutrophil percentages and a statistically significant decrease (P < 0.01) in the average lymphocyte percentages were demonstrated by the Fisher-Student "t" test (5). By the 25th day there was a reversal of the usual neutrophil to lymphocyte ratio (Chart 2). The average monocyte and eosinophil percentages were not significantly altered during the course of this study.

Chart 3 shows the bone marrow response to the injected necrotic material. From the 25th day to the conclusion of the experiment, the average percentage of young neutrophils showed a statistically significant increase (P < 0.01). Neutrophils with "toxic" granules or cytoplasmic vacuoles, noted during the ulcerated phase of sarcoma growth in the hamster cheek pouch (12), were not seen. In about 20 per cent of these hamsters occasional metamyelocytes and rare myelocytes were seen in the peripheral blood after 40 days.

The sedimentation rate increased in the animals that received injections of necrotic material, but showed no significant change in the control group (Chart 4).

The animals that were given injections of sterile, cell-free extract of apparently viable tumor failed to show a leukocytosis, a reversal of the usual neutrophil to lymphocyte ratio, or an elevation in sedimentation rate.

Gross autopsy examination of experimental and control animals was essentially negative. The bone marrow of animals in the experimental groups generally showed a slight apparent increase in
cellularity and hyperplasia of the erythrocytic and granulocytic series after 50 days. The marrow of control animals showed only a slight hyperplasia of the erythrocytic series.

Blood cultures on the three groups of animals were negative.

**DISCUSSION**

The results of this study show that the same alterations in blood cell factors previously reported in hamsters bearing transplantable, methylcholanthrene-induced sarcomas can be produced in non-tumor-bearing hamsters by the injection of a sterile, cell-free, saline suspension of necrotic tumor tissue. Thus, it would appear that the changes in sedimentation rates and in total and differential percentage of immature neutrophils in the hamster cheek pouch without evidence of external ulceration, while not evident on gross examination of the tumor in situ, are readily identifiable on the cut surfaces of small hamster sarcomas (11).

Other investigators have noted increases in leukocytes and neutrophils and decreases in lymphocytes in other experimental animals bearing tumors (1–4, 6, 8–10, 13). These changes have been attributed to the particular tumor studied (1, 6), to protein substances derived from the tumor which passed into the blood and stimulated the bone marrow of the cancer-bearing animal (13), to tumor size without determination of the presence or absence of internal tumor necrosis (2, 4), and to unknown substances originating in the local sarcoma growth (10). Perhaps in some of these instances, as in the hamster, the alterations in white blood cells are also due to the products of necrosis of the tumor and not to the tumor per se.

**SUMMARY**

1. Sterile, cell-free, saline extracts of necrotic portions of methylcholanthrene-induced hamster sarcoma injected into hamsters produced a leukocytosis, neutrophilia, increase in young neutrophils, white blood cell counts in sarcoma-bearing hamsters occurring when the tumors become necrotic are dependent on the presence of necrosis within the tumor rather than on the presence of the tumor tissue itself.

The rise in leukocytes and polymorphonuclear cells which begins before there is gross evidence of cheek pouch ulceration or tumor necrosis is presumably the resultant when focal necrosis within the tumor substance occurs. Such foci of necrosis.
a reversal of the normal lymphocyte to neutrophil ratio, and an elevated sedimentation rate.

2. The white blood cell and sedimentation rate changes noted are comparable to those observed during sarcoma growth.

3. It was concluded that the necrosis produced during sarcoma growth in the hamster cheek pouch was responsible for alterations in the leukocyte values and sedimentation rates.

4. Sterile cell-free extracts of viable sarcoma injected into hamsters failed to produce alterations in the white blood cells or the sedimentation rate.

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